Stem Cell Research 43 (2020) 101726

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Non-invasively enhanced intracranial transplantation of mesenchymal stem cells using focused ultrasound mediated by overexpression of cell-adhesion molecules

Jihyeon Lee[a,b,†], Won Seok Chang[b,†,*], Jaewoo Shin[a,b], Younghee Seo[a,b], Chanho Kong[b], Byeong-Wook Song[c,d], Young Cheol Na[e], Bong Soo Kim[c,d], Jin Woo Chang[a,b,*]

[a] Brain Korea 21 PLUS Project for Medical Science & Brain Research Institute, Yonsei University College of Medicine, Seoul 03722, Republic of Korea
[b] Department of Neurosurgery, Yonsei University College of Medicine, Seoul 03722, Republic of Korea
[c] Biomedical Research Institute, International St. Mary's Hospital, Incheon Metropolitan City 22711, Republic of Korea
[d] Department of Medical Science, Catholic Kwandong University College of Medicine, Gangneung 25601, Republic of Korea
[e] Department of Neurosurgery, Catholic Kwandong University College of Medicine, International St. Mary's Hospital, Incheon Metropolitan City 22711, Republic of Korea

ARTICLE INFO

Keywords:
Stem cell transplantation
Mesenchymal stem cells
Cell adhesion molecules
Cell migration
Cytokines
Stem cell-microenvironment interactions

ABSTRACT

Although there have been reports of promising results regarding the transplantation of mesenchymal stem cells (MSCs) for neurodegenerative diseases through the use of neuronal differentiation or control of the microenvironment, traditional surgical transplantation methods like parenchymal or intravenous injection have limitations such as secondary injuries in the brain, infection, and low survival rate of stem cells in the target site. Focused ultrasound (FUS) treatment is an emerging modality for the treatment of brain diseases, including neurodegenerative disorders. The various biological effects of FUS treatment have been investigated; therefore, the goal is now to improve the delivery efficiency and function of MSCs by capitalizing on the advantages of FUS. In this study, we demonstrated that FUS increases MSC transplantation into brain tissue by >2-fold, and that this finding might be related to the activation of intercellular adhesion molecule-1 in endothelial and subendothelial cells and vascular adhesion molecule-1 in endothelial cells.

KEY resources table

Reagent or resource	Source	Identifier
Antibodies		
Mouse monoclonal anti-ICAM-1	Santa cruz biotechnology	sc-8439
Mouse monoclonal anti-VCAM-1	Santa cruz biotechnology	sc-13,160
Rabbit monoclonal anti-Iba1	Wako chemicals	019–19,741
Rabbit polyclonal anti-GFAP	Abcam	ab116010
Mouse monoclonal anti-Rat RECA-1	Bio-rad	MCA970R
Experimental Models: Cell Lines		
Bone marrow-derived mesenchymal stem cells	Stem Cell Res Ther. 2015 Sep 15;6:174.	N/A
Other		
Definity	Lantheus Medical Imaging	N/A

1. Introduction

Diseases of the central nervous system (CNS), such as Alzheimer's disease (AD) and Parkinson's disease (PD), usually result in degeneration and irreversible damage to the structures and functions of the brain, which is often accompanied by serious cognitive or physical impairments. Many types of novel therapeutic modalities, including targeted medicine (Danon et al., 2019), deep brain stimulation (Lozano et al., 2019), radiosurgery (Jang et al., 2015), and stem cell treatment (McLauchlan and Robertson, 2018), have been evaluated for the treatment of patients with CNS diseases (Chang and Chang, 2017); however, there remains no consensus regarding the choice of effective therapy for neurodegenerative diseases.

Stem cell therapy represents a promising treatment modality for CNS diseases, with positive outcomes and the feasibility of the technique being confirmed in recent years (Kamelska-Sadowska et al.,

Abbreviations: FUS, focused ultrasound; BM-MSC, bone marrow derived-mesenchymal stem cell; ICAM-1, intercellular adhesion molecules 1; VCAM-1, Vascular cell adhesion molecules 1; RECA-1, Rat endothelial cell antigen 1; MB, Microbubble

* Corresponding authors.
E-mail addresses: changws0716@yuhs.ac (W.S. Chang), jchang@yuhs.ac (J.W. Chang).
† Jihyeon Lee and Won Seok Chang contributed equally to this work.

https://doi.org/10.1016/j.scr.2020.101726
Received 24 April 2019; Received in revised form 16 January 2020; Accepted 29 January 2020
Available online 30 January 2020

2019). Early in their development, stem cell applications focused on the replacement of damaged neural structures with neural stem cells (NSCs). One of the goals of NSC therapy is the replacement of dopaminergic neurons in PD, which is characterized by progressive degeneration of dopaminergic neurons in substantia nigra compacta, and many preclinical and clinical trials have been conducted for this purpose (Trounson et al., 2011). Previous studies reported that human NSCs (hNSCs) completely restored and ameliorated the functional defects in 6-hydroxydopamine-induced Parkinsonian mice (Zuo et al., 2017), and that autologous NSCs induced motor recovery and increased dopamine uptake in the transplanted putamen of a patient (Lévesque et al., 2009).

Although these results show acceptable outcomes, a few concerns have also been raised, such as the reduced efficacy over long-term use, surgical risks during transplantation, and the cost of treatment (de Munter et al., 2014). Moreover, the application of NSC therapy to treat CNS diseases has other limitations. Because transplanted NSCs rarely make connections with existing neurons, results show poor functional improvement, even after NSC treatment (Abeysinghe et al., 2016). In addition, following the intravenous injection of NSC, a number of NSCs cannot pass through the lungs, resulting in only a small number of NSCs reaching their target area in the brain (Fischer et al., 2009).

Mesenchymal stem cells (MSCs) have emerged as a possible candidate for stem cell therapy for CNS diseases (Azari et al., 2010). MSCs exhibit various types of biological effects, including neuroprotective and immunomodulatory effects (Cabanes et al., 2007). Because most neurodegenerative disorders are accompanied by immune dysfunction, MSCs are proposed as a potential candidate for the treatment of neurodegenerative disorders (Abeysinghe et al., 2016). Previous studies reported that MSCs reduced amyloid-β plaques in an AD mouse model by secreting soluble intracellular adhesion molecule-1 (ICAM-1) (Kim et al., 2011) and increased neurogenesis in the subventricular zone and differentiation of neural precursor cells into dopaminergic neurons in the substantia nigra in a PD model (H.J. Park et al., 2012). Additionally, clinical trials support the therapeutic efficacy of MSCs in PD, stroke, and multiple system atrophy (Buckner, 2010; Honmou et al., 2011; Venkataramana et al., 2010). However, the application of MSCs also has limitations, especially regarding transplantation modalities related to poor efficacy of delivery and survival rates.

Recently, a new treatment for CNS diseases by using focused ultrasound (FUS) was developed. High-intensity FUS allows thermal ablation of the target area into which the FUS energy is concentrated. However, low-intensity FUS has multiple biological effects, including blood-brain barrier (BBB) modulation, neuromodulation, immune modulation, and changes to the cerebral microenvironment (Chang and Chang, 2017; Curley et al., 2017; Kubanek, 2018; Shin et al., 2018). Although a study suggested enhancing FUS-mediated trans-endothelial migration of NSCs by using microbubbles (MBs) (Burgess et al., 2011), the impact of the BBB during cell migration remains unknown, and the effects of applying FUS with stem cells have only been explored at an early experimental stage.

Therefore, this study aimed to determine whether non-invasive FUS can facilitate the migration of MSCs to a target area in the brain and investigated the in vivo effects of adhesion-molecule alterations induced by low-intensity FUS in a rat model.

2. Materials and methods

2.1. Bone marrow-derived (BM)-MSC preparation

MSCs were purified, as previously described (Song et al., 2015). Briefly, BM-MSCs were harvested from normal rat bone marrow and maintained at 37 ˚C and 5% CO_2. The culture medium used was Dulbecco's modified Eagle medium (DMEM)-low glucose supplemented with 10% fetal bovine serum(FBS)(Invitrogen, Carlsbad, CA, USA), 100 U/mL penicillin (Invitrogen), and 100 μg/mL streptomycin (Invitrogen). The medium was replaced every 2 or 3 days, and we used BM-MSCs at the 5th passage. Cells at this passage were prestained using the PKH26 red fluorescent cell linker kit (Sigma-Aldrich, St. Louis, MO, USA) to identify PKH26 bound to the cell membrane.

2.2. Animals and MSC transplantation with FUS

All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of Yonsei University, Korea (IACUC number: 2018–0167). Rats were housed in groups of three per cage in a temperature/humidity-controlled room with a 12-/12-h light/dark cycle and with access to food and water ad libitum. Every effort was made to minimize the number of rats used and overall animal suffering.

Thirty-three male Sprague–Dawley rats (200–220 g) were randomly assigned to one of five experimental groups before sonication. Rats in the normal group ($n = 5$) did not undergo any surgical procedures, and those in the IV group ($n = 7$) underwent stem cell transplantation via the tail vein without any surgical procedures. All sonication groups ($n = 21$) were bilaterally sonicated with low-intensity FUS energy in the lateral hippocampal area, with seven rats also receiving tail-vein injection of BM-MSCs after 3 h. Rats in the FUS only group ($n = 9$) underwent sonication only, and these rats were sacrificed 3 h after sonication in order to confirm the expression of adhesion molecules. The remaining rats ($n = 5$) were injected with Evans blue via the tail vein 3 h after sonication to confirm BBB permeability at the time when MSCs were infused.

Animals were deeply anesthetized with a mixture of ketamine (75 mg/kg), acepromazine (0.75 mg/kg), and xylazine (4 mg/kg) and secured in a stereotaxic frame. An FUS beam was targeted to the bilateral hippocampal region (AP −3.5; ML ±2) using a 3D positioning system. Definity (mean diameter range: 1.1–3.3 μm; Lantheus Medical Imaging, North Billerica, MA, USA) MBs were diluted in saline and injected intravenously into the tail vein 10 s prior to ultrasound sonication. Evans blue (2%, 100 mg/kg) was injected intravenously at 3 h post-sonication in the selected rats ($n = 5$), which were sacrificed 30 min later to examine BBB permeability.

Three hours after sonication, seven rats in the FUS + Cell group received 200 μL of BM-MSCs (3×10^6 cells/200 μL) via tail-vein injection. Another seven rats in the IV group received 200 μL of BM-MSCs (3×10^6 cells/200 μL) via tail-vein injection only. All rats were immunosuppressed with cyclosporine (12.5 mg/kg via daily intraperitoneal injection) starting the day before transplantation and continuing up to the day they were sacrificed. These 14 rats were sacrificed at 24 h after stem cell transplantation.

2.3. FUS preparation and sonication parameters

FUS setup and sonication parameters were determined based on a previous study (Shin et al., 2018). A single-element spherically focused transducer (center frequency: 515 KHz; third harmonic: 1.6 MHz; focal depth: 51.7 mm; and radius of curvature: 63.2 mm; H-107MR; Sonic Concept Inc., Bothell, WA, USA) was driven by a waveform generator (33220A: Agilent Technologies, Palo Alto, CA, USA) and radio-frequency power amplifier (240 L; ENI Inc., Rochester, NY, USA). The transducer electrical impedance was matched to the output impedance of the amplifier (50 ΩS with an external matching network (Sonic Concept Inc.). A cone filled with distilled and degassed water was mounted onto the transducer assembly, and a needle-type hydrophone (HNA-0400; Onda, Sunnyvale, CA, USA) was used for transducer calibration to measure the acoustic beam profile in the tank filled with degassed water. The sonication parameters involved a 10-ms burst duration at a 1-Hz pulse-repetition frequency for a total duration of 300 s to generate average peak-negative pressures of 0.25 MPa

2.4. Histopathologic analysis

All animals were anesthetized and perfused with normal saline and cold 4% paraformaldehyde. The brains were stored in 4% paraformaldehyde (Duksan, Seoul, South Korea) for 3 days at 4 ˚C and transferred to 30% sucrose (Duksan) for 3 days. The brains were then cut into 30-μm coronal sections using a freezing microtome (Leica Biosystems, Wetzlar, Germany) and stored in cryoprotectant solution consisting of 0.1 M phosphate buffer (pH 7.2), 30% sucrose, 1% polyvinylpyrrolidone (Sigma-Aldrich), and 30% ethylene glycol (Thermo Fisher Scientific, Rockford, IL, USA) at −20 ˚C. Fluorescence immunohistochemistry was performed to detect ICAM-1, vascular cell-adhesion molecule (VCAM-1), glial cells, and endothelial cells. Sections were blocked with 5% normal goat serum (Vector Labs, Burlingame, CA, USA) and incubated with primary antibodies at the following dilutions: ICAM-1 (sc-8439; Alexa Fluor 488; 1:100; Santa Cruz Biotechnology, Dallas, TX, USA), VCAM-1 (sc-13,160; Alexa Fluor 647; 1:100; Santa Cruz Biotechnology), microglia (Iba1; 019–19,747; 1:300; Wako Chemicals, Richmond, VA, USA), astrocytes (glial fibrillary acidic protein; ab116010; 1:300; Abcam, Cambridge, UK), and endothelial cells (RECA-1; MCA970R; 1:300; Serotec, Oxford, UK). After the primary immunoreaction, sections were incubated with secondary antibodies conjugated with Alexa Fluor 647 (AF647; A20991; 1:300; Thermo Fisher Scientific) or Alexa Fluor 488 (AF488; A11001; 1:300; Thermo Fisher Scientific). The staining intensity of the sections was visualized with an LSM 700 confocal microscope (Carl Zeiss, Jena, Germany).

Hematoxylin (Vector Labs) & eosin (Sigma-Aldrich) (H&E) staining was performed to examine brain-tissue damage and red blood-cell extravasation. Brains were processed for paraffin-wax embedding and cut into 4-μm sections, which were stained with H&E.

The BBB opening was confirmed by histological assessment of the gross tissue sections and the presence of Evans blue extravasation in the FUS-sonicated region of the brain.

2.5. Statistical analysis

All data are expressed as the mean ± standard error of the mean (SEM). Because counting data were not normally distributed (D'Agostino and Pearson omnibus normality test: the N was too small), nonparametric analysis was performed using the two-tailed non-parametric Mann–Whitney test. SPSS (v.22.0; IBM Corp. Armonk, NY, USA) was used for statistical analysis, and a $p < 0.05$ was considered statistically significant.

2.6. Key resource table

Other details for reagents and chemicals used in this study are detailed in the key resource table.

3. Results

3.1. Effects of FUS on enhancing MSC trans-endothelial migration

To analyze the effects of FUS on trans-endothelial MSC migration, we compared five rats not receiving FUS (control) with seven rats receiving FUS after injection of PKH26-prestained MSCs (FUS group) via the tail vein. The whole hippocampal region, which was the FUS-target area, was transected from the anterior end to the posterior end with a 30-μm width in both groups, and PKH26-positive cells in each coronal section were counted and summed for comparison. The mean number of PHK26-positive cells in the hippocampal region in the control group was 739.6 ± 104.0, whereas that in the FUS group was 1700.7 ± 111.8 ($p = 0.02$), indicating that FUS significantly increased the yield of trans-endothelial migrated MSCs (Fig. 1).

3.2. Effect of FUS on ICAM-1 and VCAM-1 expression

Five rats without any intervention (control) and nine rats receiving FUS sonication in the hippocampal region (FUS group) were used to analyze the effect of FUS on ICAM-1 and VCAM-1 expression. The same three slices of the hippocampal region in both groups were selected for immunohistochemistry, and cells reactive to antibodies for ICAM-1 and VCAM-1 were counted for comparison. The mean number of ICAM-1-positive cells in the control group was significantly lower than that in the FUS group (167.5 ± 30.7 vs. 356.9 ± 57.6, respectively; $p = 0.003$). Similarly, the mean number of VCAM-1-positive cells in the control group was significantly lower than that in the FUS group (199.9 ± 27.0 vs. 335.0 ± 44.7, respectively; $p = 0.003$) (Fig. 2).

Analyses of ICAM-1 and VCAM-1 co-localization with other cells, such as glial cells and microglia, were performed in the dentate gyrus, hilus of hippocampus, cornu ammonis (CA)1, and CA3. The results showed that ICAM-1 was expressed in both glial cells and microglia, whereas VCAM-1 expression was mainly observed in endothelial cells across all hippocampal structures (Fig. 3).

3.3. Microglial activities after FUS

Microglia are a type of glial cell located throughout the CNS, accounting for 10–15% of all cells found within the brain (Xavier et al., 2014). As resident macrophages, they act as the first and main form of active immune defense in the CNS (Filiano et al., 2015). Because microglia are mainly associated with the immune response, we determined whether there were changes in microglia after FUS. As shown in Fig. 4, microglial activities were markedly increased after FUS. Evaluation of damage to the brain region by H&E staining revealed no visible structural injuries or extravasated red blood cells in sonicated brain regions (Fig. 5), indicating that FUS induced an inflammatory reaction without structural damage.

3.4. Evans blue extravasation at the time when MSC was infused

To confirm BBB permeability when MSC was injected, we examined five rats injected with Evans blue 3 h after FUS sonication. Leakage of Evans blue was visualized in the sonicated hippocampal region. To show the dye leakage obviously, Evans blue fluorescence (excitation at 620 nm, emission at 680 nm) was detected in cryostat sections of brain tissue using LSM 700 confocal microscopic imaging.

4. Discussion

In this study, we focused on the biological effect of FUS in regard to stem cell homing and determined whether FUS sonication at a specific area of the brain could non-invasively increase the efficacy of stem cell transplantation. In the FUS-treated group, we observed $a > 2$-fold increase in MSC migration relative to that observed in the untreated group and verified the upregulated expression of cell-adhesion molecules (CAMs) ICAM-1 and VCAM-1 in the FUS treated group. Interestingly, both ICAM-1 and VCAM-1 were expressed in endothelial cells, whereas only ICAM-1 was expressed in subendothelial cells, such as astrocytes and microglia, even without structural injury and hemorrhage. A previous study reported that the activation of CAMs is related to stimulation by various cytokines, and that stem cells migrate to the activated site (Nitzsche et al., 2017). Therefore, our results suggest that increased FUS-induced expression of ICAM-1 and VCAM-1 could be associated with the targeted homing of MSCs, although further studies are required to confirm this finding.

4.1. Non-invasive and targeted MSC transplantation into the brain using FUS

Many clinical trials focused on the treatment of CNS diseases using

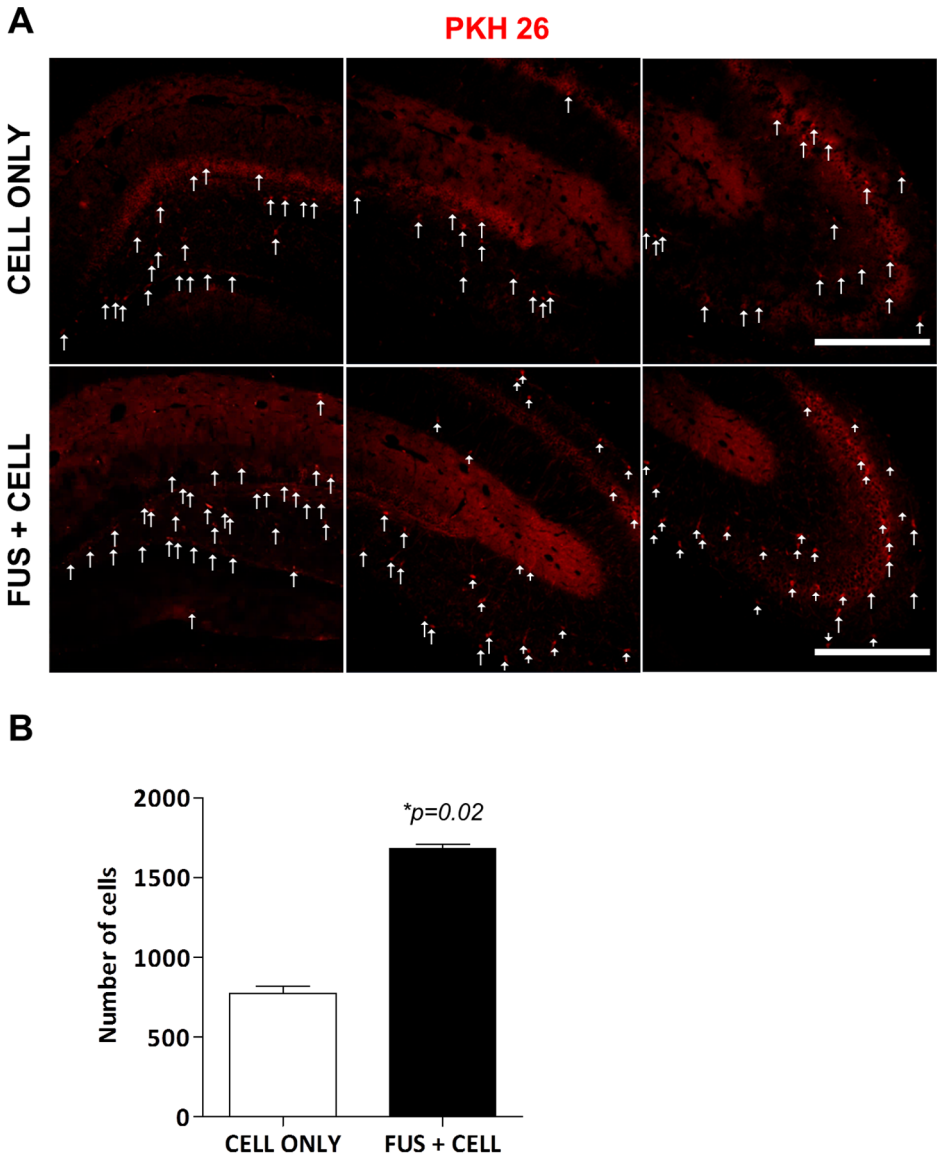

Fig. 1. Comparison of transplantation yield between the control and FUS groups. Immunohistochemistry results of PKH26-positive cells in the hippocampal region (A) and statistical analysis of the difference between groups (B) 'Cell only' indicates the control group, and "Cell + FUS" indicates the group receiving cell transplantation with FUS. Data are expressed as the mean ± SEM.

stem cells have been conducted; however, few have reported successful outcomes, despite preclinical studies for each disease model showing excellent therapeutic effects. These unfavorable results could be associated with various factors, including the number of transplanted stem cells, biological properties of each stem cell, and route of transplantation. Given that the number of stem cells in the target area is a critical factor for successful treatment, invasive surgical methods, including direct transplantation and transplantation using a catheter and reservoir implantation, have been used (Baek et al., 2012; Park et al., 2018). However, because these modalities require direct injection or implantation into the brain parenchyma, surgical complications may occur.

Other strategies to improve MSC homing include modulation of stem cells to exhibit reactivity to migratory stimuli and alteration of target sites to attract stem cells (Naderi-Meshkin et al., 2015). These strategies include treatment of MSCs with chemical compounds (Tsai et al., 2011), preconditioning with hypoxia (Liu et al., 2012), genetic modifications (Bobis-Wozowicz et al., 2011), and coating with antibodies (Gundlach et al., 2011). Additionally, target-site modulation to attract MSCs involves direct injection of chemokines (Segers et al., 2007) and direct transfection of target tissue with chemokine-encoding genes (Penn et al., 2013). However, these methods have limitations. Treatment with various factors or genetic alteration of MSCs can cause unwanted side effects, including degradation of MSC function and angioma formation (Phillips and Tang, 2008; Udalamaththa et al., 2016), and modulation of the target site through direct application into the tissue is still invasive.

Apart from the previously mentioned somewhat invasive and inefficient methods, magnetic guidance (Yun et al., 2018) and radiotherapy (Mouiseddine et al., 2007), are two relatively non-invasive methods that have been developed. However, these methods also have limitations in terms of targeted delivery, as magnetic guidance cannot reach deep areas of the brain, and radiotherapy can cause unnecessary tissue injuries. Additionally, when applying stem cell therapies to the brain, the BBB can hamper the entry of stem cells into the CNS (de Munter et al., 2014). Several studies have utilized FUS as a means to overcome these limitations.

Previous studies evaluated the effect of FUS on stem cell application in various organs. Ghanem et al. applied FUS to a rat model of acute myocardial infarction to promote stem cell grafting in a "proof of

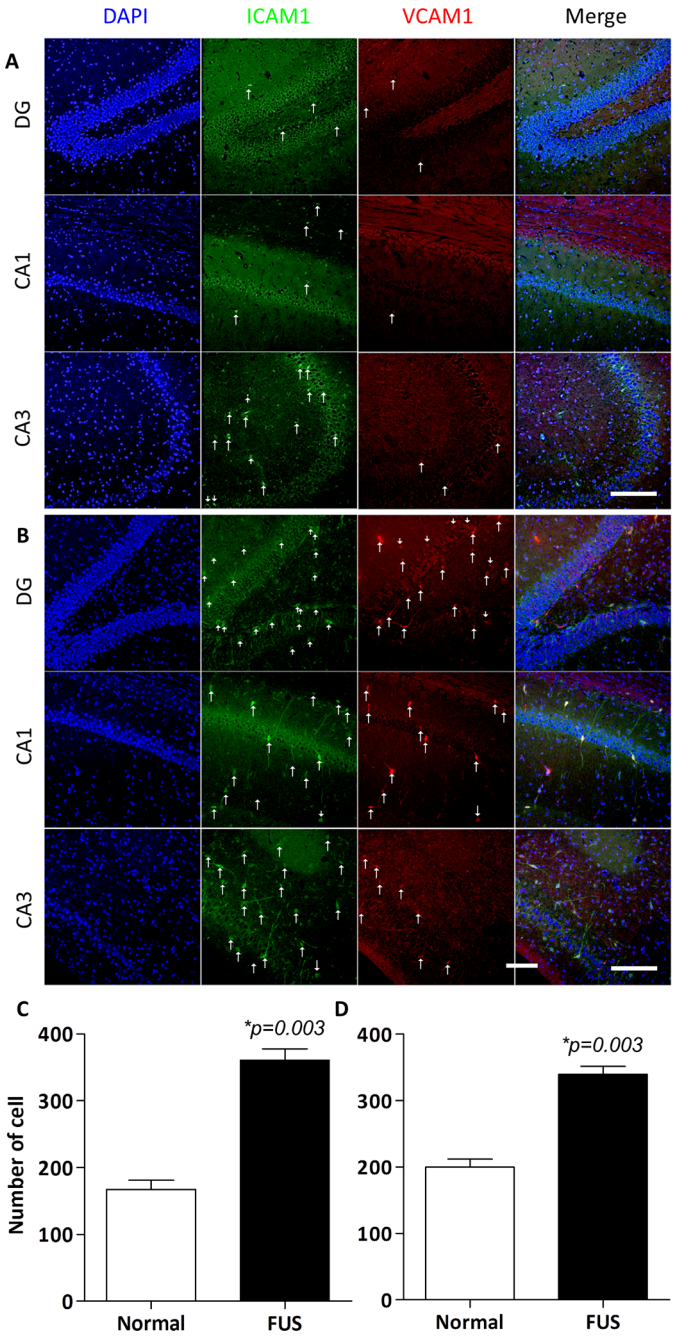

Fig. 2. Immunohistochemical analysis of ICAM-1 and VCAM-1. Expression of both ICAM-1 and VCAM-1 was higher in FUS-treated rats (B) than in untreated rats (A) Comparison of ICAM-1(C) and VCAM-1(D) between the control and FUS groups. Data are expressed as the mean ± SEM.

concept" study involving intravenous transplantation of MSCs, resulting in cell numbers in the myocardium ~50% to 60% higher in the FUS group than in the untreated group (Ghanem et al., 2009). Furthermore, they found that the activities of cytokines, such as interleukin (IL) – 1β, tumor necrosis factor (TNF)-α, and matrix metalloproteinase, increased in the FUS-treated group, and this effect was possibly related to the increased trans-endothelial migration of MSCs. Another study reported the use of FUS without MBs for targeted homing of stem cells (Burks et al., 2013), where intravenous injection of MSCs and endothelial precursor cells along with FUS treatment was applied to skeletal muscle. The results indicated achievement of maximal stem cell delivery when FUS was performed before cell infusion, and this was possibly due to the increased activities of chemo-attractants. Interestingly, they observed increased stem cell homing when FUS treatment and cell infusion were repeated daily, which indicated that the number of targeted cells can be modulated and substantially increased over short periods of time, regardless of the type of stem cell.

Recently, simultaneous application of FUS treatment with MB and stem cell transplantation into the brain was investigated (Burgess et al., 2011). The histological and immunohistochemical results obtained after FUS treatment of the brain prior to NSC transplantation indicated the presence of living NSCs in the targeted area of the brain. Limited tissue damage was confirmed by H&E staining that showed red blood cell extravasation. Although the vascular microstructure in the brain differs from that in other organs due to the unique microstructure of the BBB, it demonstrated the efficacy of FUS-mediated inducement of stem cell homing in the brain.

In the present study, we determined whether FUS treatment with MB could enhance MSC homing to the target area without causing structural brain injury such as red blood cell extravasation. FUS treatment was targeted to the hippocampus region, because the hippocampus is a potential target for cognitive-related disorders, which represents one of the major indications of MSC treatment (Cho et al., 2018). We used BM-MSCs without any pretreatment, such as hypoxic preconditioning or treatment with chemical compounds and in the absence of any factor that could possibly artificially modulate the microenvironment of the target site. Burgess et al. delivered NSC to a rat brain using FUS with MB in acutely, resulting in NSC migration to the parenchyma (Burgess et al., 2011). In the present study, we applied MSCs to a rat brain using FUS with MB 3 h after sonication. Counting of MSCs in the whole hippocampus in both groups showed $a > 2$-fold higher average number of MSCs in the hippocampus in the FUS-treated group than in the untreated group (Fig. 1; $p = 0.002$). This result suggests the possibility that the application of ultrasound can efficiently transfer other stem cells through the BBB to the brain parenchyma. Alkins et al. evaluated whether FUS treatment could increase immune-cell migration into the targeted area of the brain, revealing that FUS treatment enhanced immune-cell migration by ~2-fold relative to that in the control group (Alkins et al., 2013). This result was comparable to the transplantation yield of MSCs with FUS-treatment in the present study and suggests that, similar to the immune-cell response to FUS treatment, FUS treatment can enhance trans-endothelial migration of MSCs into brain tissue.

Our findings address the limitations of invasiveness and low-delivery efficiency of transplantation methods and demonstrated a method for enhancing MSC homing noninvasively with higher efficacy to desired brain regions.

4.2. Role of CAMs and microglia activation in FUS-mediated targeted MSC transplantation into the brain

Stem cells exhibit a homing response to injured tissues mediated by the increased expression of chemokine receptors or enhanced secretion of chemokines by the injured tissue (Karp and Leng Teo, 2009). FUS can not only selectively focus activity to specific areas of the tissue but can also target deep structures using magnetic resonance image technology without affecting other tissues. At the target region, the mechanical pressure applied by sound waves induces various biological effects. Previous studies demonstrated that FUS sonication with MBs to the brain can cause a sterile inflammatory response by upregulating a variety of inflammatory and trophic factors (Kovacs et al., 2017) (McMahon and Hynynen, 2017); however, MSC homing to the brain has not yet been assessed using this method. IL-1β or TNF-α are potentially related to the chemotactic migration of MSCs toward the endothelium and across matrix barriers according to a myocardial infarction model (Ghanem et al., 2009; Segers et al., 2006). However, aside from the trans-endothelial migration induced by chemotactic agents, the tight attachment of MSCs to the endothelium under high-

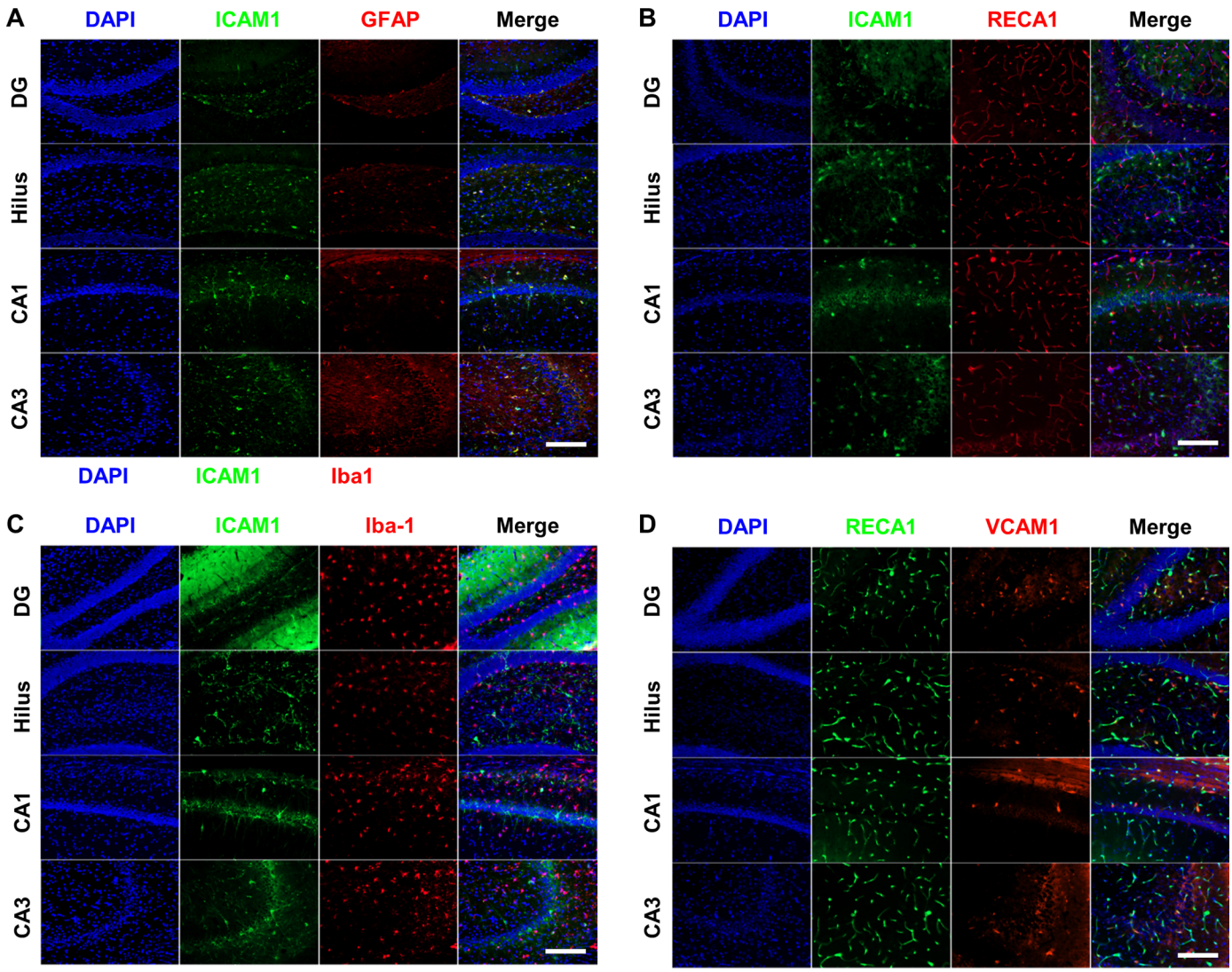

Fig. 3. CAM co-localization. Co-localization of ICAM-1-positive cells and astrocytes (A), endothelial cells (B), microglia (C) and VCAM-1 cells and endothelial cells (D). Scale bar, 200 μm.

flow conditions is also critical for targeted MSC transplantation. Here, we determined whether the expression of CAMs was enhanced following FUS treatment. The endothelium of the target tissue can possess a molecular signature that helps determine the specificity of the local immune response through binding to homing receptors, such as VCAM-1 and ICAM-1, when MSCs are delivered systemically (Dominguez et al., 2015). Moreover, coating MSCs with antibodies to ICAM-1 can promote MSC attachment to endothelial cells in vitro under high-flow conditions (Ko et al., 2009). Therefore, we confirmed the ICAM-1 and VCAM-1 levels, finding marked FUS-induced increases in ICAM-1 expression in endothelial cells (Figs. 2 and 3), which possibly indicates a mechanism of targeted MSC transplantation, where FUS induces MSC binding to endothelial cells in the target area. Interestingly, ICAM-1 expression in subendothelial cells, such as astrocytes, also increased (Fig. 2 and 3). ICAM-1 in subendothelial cells plays a role in transporting drugs across the BBB (Hsu et al., 2014); therefore, this result suggested that increased ICAM-1 levels in subendothelial cells also affect targeted MSC transplantation. Additional studies, however, are needed to clarify this conclusion.

Regarding why ICAM-1 was activated by FUS, various cytokine stimulations are related to ICAM-1 activation, and disease-like conditions can result in ICAM-1 overexpression (Hsu et al., 2014). In the present study, microglia were activated by FUS, even in the absence of structural injury (Fig. 4), indicating that FUS induced disease-like conditions without structural damage. Moreover, this might be a reason for ICAM-1 overexpression and enhanced MSC trans-endothelial migration.

Additionally, VCAM-1 expression in endothelial cells was elevated by FUS treatment (Figs. 2 and 3). VCAM-1 can enhance stem cell homing because of its propensity to make vessels resistant to detachment (Chigaev and Sklar, 2012). A previous study of the systemic delivery of MSCs in mice with inflammatory bowel disease (IBD) showed that coating MSCs with antibodies against VCAM-1 increased their efficacy to improve IBD (Ko et al., 2010). The increased expression of ICAM-1 and VCAM-1 induced by FUS might be related to the targeted homing of MSCs, although further studies are required to confirm this.

4.3. Safety issues associated with using FUS for MSC delivery

There are several possible issues associated with using FUS treatment for MSC transplantation. First, the safety of the FUS treatment should be guaranteed before being applied in humans. In this study, we selected the sonication parameters using MBs based on the results from a previous experiment by our group, and we verified the absence of FUS-induced injury with microscopic examination (Fig. 5). ICAM-1 and VCAM-1 are related to the inflammatory response (Hua, 2013;

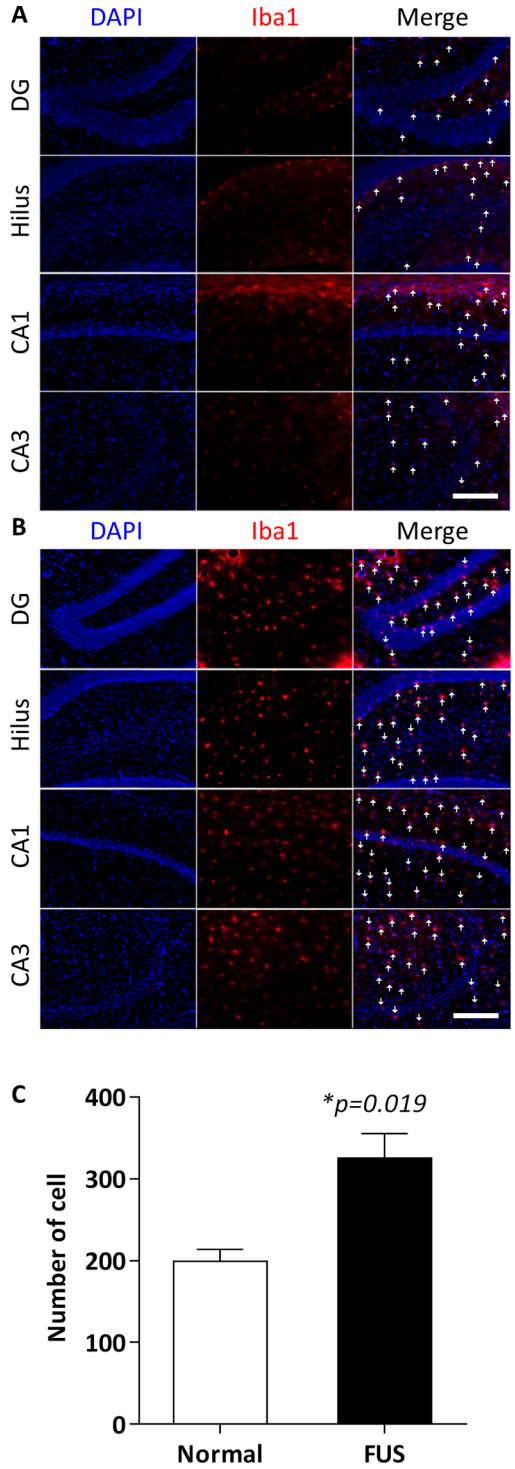

Fig. 4. FUS-induced alteration of microglial activity. Changes in microglial activity after FUS (A, B) and statistical analysis of differences between groups (C). Microglial activity was markedly increased in the FUS group (B) relative to the control group (A). Scale bar, 200 μm. Data are expressed as the mean ± SEM.

Muro and Muzykantov, 2005), and a previous study reported a tendency for MSCs to migrate to injured/inflammatory sites (Marquez-Curtis and Janowska-Wieczorek, 2013). In the present study, we showed elevations in ICAM-1 and VCAM-1 levels in FUS-sonicated regions, implying that the inflammatory reaction is induced by FUS in the targeted brain area. Additionally, Fig. 1 shows the quantitative results indicating increased migration of stem cells to the target site. Therefore, our results represent indirect evidence confirming the enhanced migration of stem cells to targeted regions induced by FUS using MBs. Although there was no extravasation of red blood cells in our acute model, it is necessary to observe long-term inflammatory responses, because repeated inflammation can cause structural changes in tissues (Downs et al., 2015).

Another issue involves the duration of BBB related to stem cell homing. Burgess et al. confirmed that NSCs cross the BBB to enter the brain parenchyma during FUS sonication (Burgess et al., 2011). Previous studies reported that the FUS-induced BBB opening persists for extended periods of time (1–10 h) (Hynynen et al., 2005; Wang et al., 2009). We experimentally confirmed through Evans blue leakage that the BBB opening was maintained 3 h after FUS sonication (Fig. 6). Our data also demonstrated enhancement in the homing of BM-MSCs to the targeted brain region (Fig. 1). We chose an MSC-infusion time based on studies describing MSC transplantation into various tissues 3 h after FUS treatment (Burks et al., 2013; Tebebi et al., 2015).

In contrast to previously published data (Burgess et al., 2011), our study parameters using the MBs did not appear to cause red blood cell extravasation (Fig. 5). Therefore, we suggest that FUS successfully promoted MSC delivery without tissue damage. However, as uncontrolled MB cavitation can damage sonicated tissue (Fan et al., 2012), the use of acoustic feedback control (e.g., passive cavitation detection) needs to be evaluated in future studies (Hua, 2013; Wu et al., 2014). Additional studies with FUS treatment alone, without MBs, are necessary to avoid the uncontrolled effects of cavitation.

5. Conclusions

FUS treatment is an emerging modality for the treatment of brain diseases, including neurodegenerative disorders. The various biological effects of FUS treatment have been investigated, and here, we determined whether FUS treatment could increase the efficacy of stem cell homing. Our results demonstrated that FUS increased MSC transplantation into brain tissue by >2-fold and this effect was possibly related to the activation of ICAM-1 in endothelial and subendothelial cells and VCAM-1 in endothelial cells. The long-term safety of FUS treatment and the combined effects of FUS treatment with other strategies for enhancing MSC homing require additional investigations.

Grant support

This study was supported by a grant from the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT, and Future Planning, Republic of Korea (2016M3C7A1914123), the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (HI19C1234) and a faculty research grant of Yonsei University College of Medicine, Republic of Korea (6–2018–0032).

CRediT authorship contribution statement

Jihyeon Lee: Conceptualization, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Won Seok Chang:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing, Funding acquisition, Supervision, Project administration. **Jaewoo Shin:** Investigation, Resources. **Younghee Seo:** Validation, Investigation. **Chanho Kong:** Investigation, Resources. **Byeong-Wook Song:** Resources, Writing - review & editing. **Young Cheol Na:** Project administration. **Bong Soo Kim:** Writing - review & editing, Project administration. **Jin Woo Chang:** Conceptualization, Funding acquisition, Supervision, Project administration.

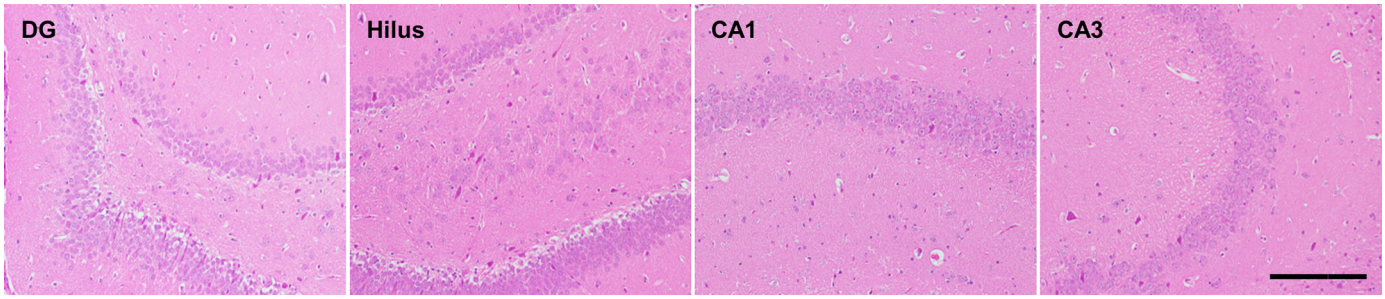

Fig. 5. H&E staining of brain tissue. Histologic evaluation was performed on sonicated brain sections. H&E staining identified.

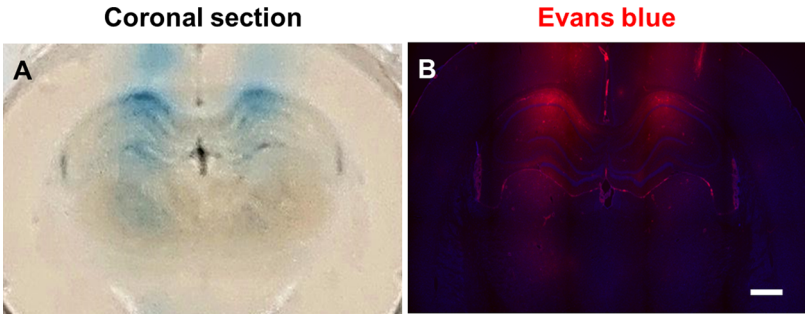

Fig. 6. Evans blue extravasation in the brain tissue 3 h post-FUS sonication. In the sonicated region, the Evans blue extravasation was confirmed in the coronal section of brain tissue (A) and the fluorescence signal of Evans blue was detected in the sonicated region (B). Scale bar, 1 mm.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to acknowledge Dong-Su Jang, MFA (Medical Illustrator, Medical Research Support Section, Yonsei University College of Medicine) for his help with our illustrations.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2020.101726.

References

Abeysinghe, H.C., Phillips, E.L., Chin-Cheng, H., Beart, P.M., Roulston, C.L., 2016. Modulating astrocyte transition after stroke to promote brain rescue and functional recovery: emerging targets include rho kinase. Int J Mol Sci 17 (3), 288.

Alkins, R., Burgess, A., Ganguly, M., Francia, G., Kerbel, R., Wels, W.S., Hynynen, K., 2013. Focused ultrasound delivers targeted immune cells to metastatic brain tumors. Cancer Res 73 (6), 1892–1899.

Azari, M.F., Mathias, L., Ozturk, E., Cram, D.S., Boyd, R.L., Petratos, S., 2010. Mesenchymal stem cells for treatment of cns injury. Curr Neuropharmacol 8 (4), 316–323.

Baek, W., Kim, Y.S., Koh, S.H., Lim, S.W., Kim, H.Y., Yi, H.J., Kim, H., 2012. Stem cell transplantation into the intraventricular space via an ommaya reservoir in a patient with amyotrophic lateral sclerosis. J Neurosurg Sci 56 (3), 261–263.

Bobis-Wozowicz, S., Miekus, K., Wybieralska, E., Jarocha, D., Zawisz, A., Madeja, Z., Majka, M., 2011. Genetically modified adipose tissue-derived mesenchymal stem cells overexpressing CXCR4 display increased motility, invasiveness, and homing to bone marrow of nod/scid mice. Exp. Hematol. 39 (6), 686–696.

Buckner, J.H., 2010. Mechanisms of impaired regulation by CD4(+)CD25(+)FOXP3(+) regulatory t cells in human autoimmune diseases. Nat. Rev. Immunol. 10 (12), 849–859.

Burgess, A., Ayala-Grosso, C.A., Ganguly, M., Jordao, J.F., Aubert, I., Hynynen, K., 2011. Targeted delivery of neural stem cells to the brain using MRI-Guided focused ultrasound to disrupt the blood-brain barrier. PLoS ONE 6 (11), e27877.

Burks, S.R., Ziadloo, A., Kim, S.J., Nguyen, B.A., Frank, J.A., 2013. Noninvasive pulsed focused ultrasound allows spatiotemporal control of targeted homing for multiple stem cell types in murine skeletal muscle and the magnitude of cell homing can be increased through repeated applications. Stem Cells 31 (11), 2551–2560.

Cabanes, C., Bonilla, S., Tabares, L., Martinez, S., 2007. Neuroprotective effect of adult hematopoietic stem cells in a mouse model of motoneuron degeneration. Neurobiol Dis 26 (2), 408–418.

Chang, W.S., Chang, J.W., 2017. Focused ultrasound treatment for central nervous system disease: neurosurgeon's perspectives. Biomed Eng Lett 7 (2), 107–114.

Chigaev, A., Sklar, L.A., 2012. Aspects of VLA-4 and LFA-1 regulation that may contribute to rolling and firm adhesion. Front Immunol 3 242-242.

Cho, J.S., Lee, J., Jeong, D.U., Kim, H.W., Chang, W.S., Moon, J., Chang, J.W., 2018. Effect of placenta-derived mesenchymal stem cells in a dementia rat model via microglial mediation: a comparison between stem cell transplant methods. Yonsei Med. J. 59 (3), 406–415.

Curley, C.T., Sheybani, N.D., Bullock, T.N., Price, R.J., 2017. Focused ultrasound immunotherapy for central nervous system pathologies: challenges and opportunities. Theranostics 7 (15), 3608–3623.

Danon, J.J., Reekie, T.A., Kassiou, M., 2019. Challenges and opportunities in central nervous system drug discovery. Trends in Chemistry.

de Munter, J.P., Melamed, E., Wolters, E.C., 2014. Stem cell grafting in parkinsonism–why, how and when. Parkinsonism Relat. Disord. 20, S150–S153.

Dominguez, G.A., Anderson, N.R., Hammer, D.A., 2015. The direction of migration of T-lymphocytes under flow depends upon which adhesion receptors are engaged. Integr Biol (Camb) 7 (3), 345–355.

Downs, M.E., Buch, A., Sierra, C., Karakatsani, M.E., Chen, S., Konofagou, E.E., Ferrera, V.P., 2015. Long-term safety of repeated blood-brain barrier opening via focused ultrasound with microbubbles in non-human primates performing a cognitive task. PLoS ONE 10 (5), e0125911.

Fan, C.H., Liu, H.L., Huang, C.Y., Ma, Y.J., Yen, T.C., Yeh, C.K., 2012. Detection of intracerebral hemorrhage and transient blood-supply shortage in focused-ultrasound-induced blood-brain barrier disruption by ultrasound imaging. Ultrasound Med Biol 38 (8), 1372–1382.

Filiano, A.J., Gadani, S.P., Kipnis, J., 2015. Interactions of innate and adaptive immunity in brain development and function. Brain Res 1617, 18–27.

Fischer, U.M., Harting, M.T., Jimenez, F., Monzon-Posadas, W.O., Xue, H., Savitz, S.I., Laine, G.A., Charles S. Cox, J., 2009. Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. 18 (5), 683–692.

Ghanem, A., Steingen, C., Brenig, F., Funcke, F., Bai, Z.Y., Hall, C., Chin, C.T., Nickenig, G., Bloch, W., Tiemann, K., 2009. Focused ultrasound-induced stimulation of microbubbles augments site-targeted engraftment of mesenchymal stem cells after acute myocardial infarction. J Mol Cell Cardiol 47 (3), 411–418.

Gundlach, C.W., Caivano, A., Cabreira-Hansen Mda, G., Gahremanpour, A., Brown, W.S., Zheng, Y., McIntyre, B.W., Willerson, J.T., Dixon, R.A., Perin, E.C., Woodside, D.G., 2011. Synthesis and evaluation of an anti-MLC1 x anti-CD90 bispecific antibody for targeting and retaining bone-marrow-derived multipotent stromal cells in infarcted myocardium. Bioconjug. Chem. 22 (8), 1706–1714.

Honmou, O., Houkin, K., Matsunaga, T., Niitsu, Y., Ishiai, S., Onodera, R., Waxman, S.G., Kocsis, J.D., 2011. Intravenous administration of auto serum-expanded autologous mesenchymal stem cells in stroke. Brain 134 (Pt 6), 1790–1807.

Hsu, J., Rappaport, J., Muro, S., 2014. Specific binding, uptake, and transport of ICAM-1-

targeted nanocarriers across endothelial and subendothelial cell components of the blood-brain barrier. Pharm Res 31 (7), 1855–1866.
Hua, S., 2013. Targeting sites of inflammation: intercellular adhesion molecule-1 as a target for novel inflammatory therapies. 4 (127).
Hynynen, K., McDannold, N., Sheikov, N.A., Jolesz, F.A., Vykhodtseva, N., 2005. Local and reversible blood–brain barrier disruption by noninvasive focused ultrasound at frequencies suitable for trans-skull sonications. Neuroimage 24 (1), 12–20.
Jang, C.K., Jung, H.H., Chang, J.H., Chang, J.W., Park, Y.G., Chang, W.S., 2015. Long-Term results of gamma knife radiosurgery for intracranial meningioma. Brain Tumor Res Treat 3 (2), 103–107.
Kamelska-Sadowska, A.M., Wojtkiewicz, J., Kowalski, I.M., 2019. Review of the current knowledge on the role of stem cell transplantation in neurorehabilitation. Biomed Res Int 2019 3290894-3290894.
Karp, J.M., Leng Teo, G.S., 2009. Mesenchymal stem cell homing: the devil is in the details. Cell Stem Cell 4 (3), 206–216.
Kim, J.Y., Kim, D.H., Kim, J.H., Lee, D., Jeon, H.B., Kwon, S.J., Kim, S.M., Yoo, Y.J., Lee, E.H., Choi, S.J., Seo, S.W., Lee, J.I., Na, D.L., Yang, Y.S., Oh, W., Chang, J.W., 2011. Soluble intracellular adhesion molecule-1 secreted by human umbilical cord blood-derived mesenchymal stem cell reduces amyloid-β plaques. Cell Death Differ. 19, 680.
Ko, I.K., Kean, T.J., Dennis, J.E., 2009. Targeting mesenchymal stem cells to activated endothelial cells. Biomaterials 30 (22), 3702–3710.
Ko, I.K., Kim, B.G., Awadallah, A., Mikulan, J., Lin, P., Letterio, J.J., Dennis, J.E., 2010. Targeting improves MSC treatment of inflammatory bowel disease. Mol Ther 18 (7), 1365–1372.
Kovacs, Z.I., Kim, S., Jikaria, N., Qureshi, F., Milo, B., Lewis, B.K., Bresler, M., Burks, S.R., Frank, J.A., 2017. Disrupting the blood-brain barrier by focused ultrasound induces sterile inflammation. Proc Natl Acad Sci U S A 114 (1), E75–e84.
Kubanek, J., 2018. Neuromodulation with transcranial focused ultrasound. Neurosurg Focus 44 (2) E14-E14.
Lévesque, M., Neuman, T., Rezak, M., 2009. Therapeutic microinjection of autologous adult human neural stem cells and differentiated neurons for parkinson's disease: five-Year post-operative outcome. Open Stem Cell J 1, 20–29.
Liu, H., Liu, S., Li, Y., Wang, X., Xue, W., Ge, G., Luo, X., 2012. The role of SDF-1-CXCR4/CXCR7 axis in the therapeutic effects of hypoxia-preconditioned mesenchymal stem cells for renal ischemia/reperfusion injury. PLoS ONE 7 (4), e34608.
Lozano, A.M., Lipsman, N., Bergman, H., Brown, P., Chabardes, S., Chang, J.W., Matthews, K., McIntyre, C.C., Schlaepfer, T.E., Schulder, M., Temel, Y., Volkmann, J., Krauss, J.K., 2019. Deep brain stimulation: current challenges and future directions. Nature Reviews Neurology 15 (3), 148–160.
Marquez-Curtis, L.A., Janowska-Wieczorek, A., 2013. Enhancing the migration ability of mesenchymal stromal cells by targeting the SDF-1/CXCR4 axis. Biomed Res Int 2013 561098-561098.
McLauchlan, D., Robertson, N.P., 2018. Stem cells in the treatment of central nervous system disease. J. Neurol. 265 (4), 984–986.
McMahon, D., Hynynen, K., 2017. Acute inflammatory response following increased blood-brain barrier permeability induced by focused ultrasound is dependent on microbubble dose. Theranostics 7 (16), 3989–4000.
Mouiseddine, M., Francois, S., Semont, A., Sache, A., Allenet, B., Mathieu, N., Frick, J., Thierry, D., Chapel, A., 2007. Human mesenchymal stem cells home specifically to radiation-injured tissues in a non-obese diabetes/severe combined immunodeficiency mouse model. Br J Radiol 80 Spec (1), S49–S55.
Muro, S., Muzykantov, V.R., 2005. Targeting of antioxidant and anti-thrombotic drugs to endothelial cell adhesion molecules. Current pharmaceutical design 11 (18), 2383–2401.
Naderi-Meshkin, H., Bahrami, A.R., Bidkhori, H.R., Mirahmadi, M., Ahmadiankia, N., 2015. Strategies to improve homing of mesenchymal stem cells for greater efficacy in stem cell therapy. Cell Biol Int 39 (1), 23–34.
Nitzsche, F., Müller, C., Lukomska, B., Jolkkonen, J., Deten, A., Boltze, J., 2017. Concise review: MSC adhesion cascade—insights into homing and transendothelial migration. 35 (6), 1446–1460.
Park, H.-J., Shin, J.Y., Lee, B.R., Kim, H.O., Lee, P.H., 2012. Mesenchymal stem cells augment neurogenesis in the subventricular zone and enhance differentiation of neural precursor cells into dopaminergic neurons in the substantia nigra of a parkinsonian model. 21 (8), 1629–1640.
Park, S.E., Lee, N.K., Na, D.L., Chang, J.W., Chang, J.W., 2018. Optimal mesenchymal stem cell delivery routes to enhance neurogenesis for the treatment of alzheimer's disease. Histology and histopathology: cellular and molecular biology 33 (6), 533–541.
Penn, M.S., Mendelsohn, F.O., Schaer, G.L., Sherman, W., Farr, M., Pastore, J., Rouy, D., Clemens, R., Aras, R., Losordo, D.W., 2013. An open-label dose escalation study to evaluate the safety of administration of nonviral stromal cell-derived factor-1 plasmid to treat symptomatic ischemic heart failure. Circ Res 112 (5), 816–825.
Phillips, M.I., Tang, Y.L., 2008. Genetic modification of stem cells for transplantation. Adv. Drug Deliv. Rev. 60 (2), 160–172.
Segers, V.F., Tokunou, T., Higgins, L.J., MacGillivray, C., Gannon, J., Lee, R.T., 2007. Local delivery of protease-resistant stromal cell derived factor-1 for stem cell recruitment after myocardial infarction. Circulation 116 (15), 1683–1692.
Segers, V.F., Van Riet, I., Andries, L.J., Lemmens, K., Demolder, M.J., De Becker, A.J., Kockx, M.M., De Keulenaer, G.W., 2006. Mesenchymal stem cell adhesion to cardiac microvascular endothelium: activators and mechanisms. Am J Physiol Heart Circ Physiol 290 (4), H1370–H1377.
Shin, J., Kong, C., Cho, J.S., Lee, J., Koh, C.S., Yoon, M.S., Na, Y.C., Chang, W.S., Chang, J.W., 2018. Focused ultrasound-mediated noninvasive blood-brain barrier modulation: preclinical examination of efficacy and safety in various sonication parameters. Neurosurg Focus 44 (2), E15.
Song, B.W., Kim, I.K., Lee, S., Choi, E., Ham, O., Lee, S.Y., Lee, C.Y., Park, J.H., Lee, J., Seo, H.H., Chang, W., Yoon, C., Hwang, K.C., 2015. 1H-pyrrole-2,5-dione-based small molecule-induced generation of mesenchymal stem cell-derived functional endothelial cells that facilitate rapid endothelialization after vascular injury. Stem Cell Res Ther 6, 174.
Tebebi, P.A., Burks, S.R., Kim, S.J., Williams, R.A., Nguyen, B.A., Venkatesh, P., Frenkel, V., Frank, J.A., 2015. Cyclooxygenase-2 or tumor necrosis factor-α inhibitors attenuate the mechanotransductive effects of pulsed focused ultrasound to suppress mesenchymal stromal cell homing to healthy and dystrophic muscle. 33 (4), 1173–1186.
Trounson, A., Thakar, R.G., Lomax, G., Gibbons, D.J.B.M., 2011. Clinical trials for stem cell therapies. 9 (1), 52.
Tsai, L.K., Wang, Z., Munasinghe, J., Leng, Y., Leeds, P., Chuang, D.M., 2011. Mesenchymal stem cells primed with valproate and lithium robustly migrate to infarcted regions and facilitate recovery in a stroke model. Stroke 42 (10), 2932–2939.
Udalamaththa, V.L., Jayasinghe, C.D., Udagama, P.V., 2016. Potential role of herbal remedies in stem cell therapy: proliferation and differentiation of human mesenchymal stromal cells. Stem Cell Res Ther 7 (1), 110.
Venkataramana, N.K., Kumar, S.K., Balaraju, S., Radhakrishnan, R.C., Bansal, A., Dixit, A., Rao, D.K., Das, M., Jan, M., Gupta, P.K., Totey, S.M., 2010. Open-labeled study of unilateral autologous bone-marrow-derived mesenchymal stem cell transplantation in parkinson's disease. Transl Res 155 (2), 62–70.
Wang, F., Cheng, Y., Mei, J., Song, Y., Yang, Y.-q., Liu, Y., Wang, Z., 2009. Focused ultrasound microbubble destruction-mediated changes in blood-brain barrier permeability assessed by contrast-enhanced magnetic resonance imaging. 28 (11), 1501–1509.
Wu, S.-Y., Tung, Y.-S., Marquet, F., Downs, M., Sanchez, C., Chen, C., Ferrera, V., Konofagou, E., 2014. Transcranial cavitation detection in primates during blood-brain barrier opening–a performance assessment study. IEEE Trans Ultrason Ferroelectr Freq Control 61 (6), 966–978.
Xavier, A.L., Menezes, J.R.L., Goldman, S.A., Nedergaard, M., 2014. Fine-tuning the central nervous system: microglial modelling of cells and synapses. Philos Trans R Soc Lond B Biol Sci 369 (1654) 20130593-20130593.
Yun, W.S., Choi, J.S., Ju, H.M., Kim, M.H., Choi, S.J., Oh, E.S., Seo, Y.J., Key, J., 2018. Enhanced homing technique of mesenchymal stem cells using iron oxide nanoparticles by magnetic attraction in olfactory-injured mouse models. Int J Mol Sci 19 (5).
Zuo, F., Xiong, F., Wang, X., Li, X., Wang, R., Ge, W., Bao, X., 2017. Intrastriatal transplantation of human neural stem cells restores the impaired subventricular zone in parkinsonian mice. Stem Cells 35 (6), 1519–1531.